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Title: One-stop separation method for extracting polyhydroxyl phytanate directly from fermented liquid

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Applicant(s) Name Inst of Microbes, Chinese Academy of Sciences
Address
Inventor(s) Name Weng Weiqi;Cai Yibin;Liu Moqing
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Abstract

A process for extracting polybydroxyl phytanate (PHAs) directly from the fermented liquid of Alcaligenes eutrophus incldues such steps as adding surfactant, sodium hypochlorite and **deforming agent to said fermented liquid to change the coagulation state of PHAs particles**, separating PHAs particles by centrifugal machine or filter, washing and baking. Its advantages are high purity up to 95% and high output rate (about 80%).
Claims

1, One kind is extracted from from fermented solution to get poly hydroxy alkane acid ester one-step isolation method directly, characterized in that the following steps make up: (1), in the fermented solution of cell comprising poly hydroxy alkane acid ester, after regulating the fermented solution PH value, add anion surface active agent, stir to react. (2), add sodium hypochlorite, deforming agent into of the above-mentioned reaction system, stir to react. (3), poly hydroxy alkane acid ester PHAs granule separating and extracting the solid phase. (4), wash with water, dry, get powder poly hydroxide alkane acid product.
2, One as described in claim 1 is extracted from from fermented solution to get poly hydroxy alkane acid ester one-step isolation method directly, characterized in that said PH value is in 5-14.
3, One as described in claim 1 is extracted from from fermented solution to get poly hydroxy alkane acid ester one-step isolation method directly, characterized in that said surfactant active is anion surface active agents such as sodium dodecyl sulphate, sodium dodecyl benzene sulfonate, Triton-100,etc..
4, One as described in claim 1 is extracted from from fermented solution to get poly hydroxy alkane acid ester one-step isolation method directly, characterized in that said surfactant active consumption is 1%-30% of poly hydroxy alkane acid ester in dried cell weight, reaction time is 0.1-24 hours.
5, One as described in claim 1 is extracted from from fermented solution to get poly hydroxy alkane acid ester one-step isolation method directly, characterized in that said deforming agent is mixed solution of the sodium hypochlorite and alkaline solution such as NaOH, KOH.
6, One as described in claim 1 is extracted from from fermented solution to get poly hydroxy alkane acid ester one-step isolation method directly, characterized in that said deforming agent reaction time is 0.5-30 minutes.
7, One as described in claim 1 is extracted from from fermented solution to get poly hydroxy alkane acid ester one-step isolation method directly, characterized in that said reaction temperature is at 10-100 ℃.
8, One as described in claim 1 is extracted from from fermented solution to get poly hydroxy alkane acid ester one-step isolation method directly, characterized in that said separating and extracting method collects the solid phase thing in order to filter or centrifugate.

One kind is extracted from from fermented solution to get poly hydroxy alkane acid ester one-step isolation method directly

This invention belongs to the technical field of bioengineering.

Poly hydroxy alkane acid ester (Poly-3-Hydroxyalkanoates, PHAs) is a kind of high molecular material that the microorganism is accumulated in cells under the uneven growing condition, it is the thermoplastic polyester that a kind of physical property is similar to chemical synthetic plastieses such as propene polymer,etc., but it has some properties that general synthetic plasticses do not possess, for instance: Biodegradability, biocompatibility, piezoelectric activity, optically active, etc, substitute one of the chemical synthetic plastics most competitive representatives. Special properties make it have extensive application prospect in such aspects as industry, agriculture, medicine, hygiene, food, study the focus paid close attention to as a lot of developed countries.

The application of PHAs has not been used in a large amount because of its great of production cost at present, and an important factor which depend on level of the production cost and industrialization is from PHAs and then extract. The back preparation method for PHAs is generally by 3 kinds: (1)Organic solvent method. The shortcoming of the this method is to need quiverful organic solvent, such as chloroform (European's Patent No. 46,017), Methylene chloride (European's Patent No. 58,480), propanone, methane,etc., with high costs, the work environment is abominable, the labour protection is expected much, extract the low yield. (2)Sodium-hypochlorite process (Biotechnology Techniques, 3 (4)s: 227-230,1989). Because sodium hypochlorite has very strong oxidizability, extract the process to make the molecular weight of PHAs influenced much, for this reason, someone proposes surfactant active-sodium-hypochlorite process (Biotechnology Techniques, 4 (4)s: 221-226,1990).Though the degradation of the this method PHAs molecular weight is obviously reduced, it need to carry on the preconditioning to cells in the process to extract, centrifugate and remove the fermented solution, and need to carry on solid-liquid separation to mixed solution in the two-step reacts, because microorganism's cell diameter is very small, need to adopt the De Laval centrifuge, it is very difficult to realize in manufacturing on a large scale, complex technology, the yield is low, high speed centrifugation is a extractive bottleneck after influencing PHAs large-scaly. And the cell concentration does not exceed 100 grams (dry weight cells) / liter in the reaction system, there is very large limitation. (3)Enzyme-surfactant active method (United States Patent 4,101,533, European's Patent No. 0145233A2). The shortcoming of the this method also lies in needing to carry on the cell preconditioning at first, and process through heat treatment of cell, enzyme and anion surface active agent is processed, could get highly purified PHAs. Complex technology, enzyme reaction condition is harsh, and need to carry on solid-liquid separation by high speed centrifugation after reacting each step too, with high costs, the project enlarges the difficulty.

The purpose of this invention lies in the back extracting method of PHAs, does not need preconditioning of cells, not needing De Laval centrifuge, and the high-density culture system to cells is applicable. The ones that utilize the general fermentation plant are ordinary

Extracting equipment, the reaction one-step of the two-step is centrifugated or filtered, can get highly purified PHAs. The craft of this invention is simple, with low costs, labor intensity is low, are extracted and had high yield, offer the condition for large-scale production.

The specific operation step of this invention is as follows:

(1)In utilizing fermented solution of cell for producing PHAs of fermentation method of microorganism, it are 5.0, 7.0, 8.0, 9.0, 10.0, 11.0, 14.0 each to regulate the fermented solution PH value with NaOH solution, wherein PH10.0 regards as and optimizes the PH value.

(2)Add anion surface active agent sodium dodecyl sulphate, sodium dodecyl benzene sulfonate, Triton-100, sodium dodecylsulphonate,etc., wherein the price of sodium dodecyl sulphate is low, extract effectually, as optimizing surfactant actives. Stir to react.

(3)**Add sodium hypochlorite, deforming agent, stir to react. Deforming agent adopts alkaline solution such as NaOH, KOH, ammonia water separately, wherein preferably NaOH solution is regarded as deforming agent.**

(4)Centrifugate or filter the above-mentioned intermixture.

(5)Wash the solid got with water and dry to get PHAs finished product.

The serviceability of this invention is strong, not merely can process the homopolymer (PHB) in poly hydroxy alkane acid ester, can process polyhydroxybutyrate-hydroxide pentanoate,etc. copolymer, and PHA content has not been required in the concentration or cell to cell of the fermented solution, can even process the cell concentration in 160 grams (dry weight) / one liter of fermentation products above fermented solution directly, it is easy to enlarge, has and has already proved on one ton of scale.

Example 1

Extract to the target: Endocellular 3 polymer-contained-fermented solution 50ml of hydroxide group butyrates (PHB)

Cell concentration: 164.4 grams (dry weight) / one liter of fermented solution

PHA content: Cells are dry and heavy 80%

The ratio to dried cell weight Central Africa PHA thing quality of the consumption of anion surface active agent: 0.2:1(w/w)

The ratio to dried cell weight Central Africa PHA thing quality of sodium hypochlorite consumption: 5:1

The specific step of this Example is as follows:

Dripping 30% NaOH solution in 50ml fermented solution regulates fermented solution PH10, add surfactant active 0.4 grams of sodium dodecyl sulphate (water and heat and dissolve, quench into), after stiring to react for 10 minutes, add chlorine bleach liquor 4.5ml and deforming agent solution 4.5ml, stir to react, separate out, go on, filter to solid 5 minute, running water wash 70 ℃ oven of the postposition dry to constant weight. The finished product passes gas chromatographic analysis

The purity is 99.5%, viscosity-average molecular weight 6.9 10 5 Da, extracts 81.8% of yield.

Example 2

Extract to the target: Endocellular 3 polymer-contained-fermented solution 100ml of hydroxide group butyrates (PHB)

Cell concentration: 107.0 grams (dry weight) / one liter of fermented solution

PHA content: Cells are dry and heavy 60.25%

The ratio to dried cell weight Central Africa PHA thing quality of the consumption of anion surface active agent: 0.5:1(w/w)

The ratio to dried cell weight Central Africa PHA thing quality of sodium hypochlorite consumption: 8:1

The specific step of this Example is as follows:

Drip 30% NaOH solution to regulate fermented solution PH10 in 100ml fermented solution, add surfactant actives after 2.3 grams of sodium dodecyl sulphate (water and heat and dissolve, quench into, react for 19 hours, add chlorine bleach liquor 21ml and deforming agent solution 16ml, stir to react and separate out and pay filtering to the solid, the running water washes 70 ℃ oven of the postposition to dry to constant weight. The finished product is 98.2% through the purity of gas chromatographic analysis, viscosity-average molecular weight 4.6 10 5 Da, extracts 78% of yield.

Example 3

Extract to the target: Endocellular 3 polymer-contained-fermented solution 50mL of hydroxide group butyrates (PHB)

Cell concentration: 164.4 grams (dry weight) / one liter of fermented solution

PHA content: Cells are dry and heavy 80%

The ratio to dried cell weight Central Africa PHA thing quality of the consumption of anion surface active agent: 0.4:1(w/w)

The ratio to dried cell weight Central Africa PHA thing quality of sodium hypochlorite consumption: 8:1

The specific step of this Example is as follows:

Adding 15% NaOH solution into 50mL fermented solution regulates fermented solution PH10, add surfactant active 0.66 grams of sodium dodecyl benzene sulfonate (water and heat dissolving and cool, quench into) into, after stiring to react for 20 minutes, add chlorine bleach liquor 8mL and deforming agent solution 8.1mL, stir to react and separate out and pay filtering to the solid, the running water washes 70 ℃ oven of the postposition to dry to constant weight. The finished product is 99.0% through the purity of gas chromatographic analysis, viscosity-average molecular weight 5.2 10 5 Da, extracts 72% of yield.

Example 4

Extract to the target: Endocellular polymer-contained 3-hydroxy-butanoic acid-3-hydroxy pentanoate (PHBV, HV

Fermented solution 100ml of 10%) with the content of PHBV

Cell concentration: 128.8 grams (dry weight) / one liter of fermented solution

PHA content: Cells are dry and heavy 80%

The ratio to dried cell weight Central Africa PHA thing quality of the consumption of anion surface active agent: 0.3:1(w/w)

The ratio to dried cell weight Central Africa PHA thing quality of sodium hypochlorite consumption: 6:1

The specific step of this Example is as follows:

Dripping 30% NaOH solution in 100ml fermented solution regulates fermented solution PH10, add surfactant active 0.8 grams of sodium dodecyl sulphate (water and heat and dissolve, quench into), after stiring to react for 10 minutes, add chlorine bleach liquor 11ml and deforming agent solution 6.0ml, stir to react, separate out, go on. filter to solid 30 minute, running water wash 70 ℃

oven of the postposition dry to constant weight. The finished product is 97% through the purity of gas chromatographic analysis. viscosity-average molecular weight 6.0 10 5 Da, extracts 85% of yield.